

Abstract

Methods, compositions, and kits for the detecting and/or quantification of polynucleotides are provided. An exemplary method provides hybridizing a first primer to the target polynucleotide and forming equal length primer extension products using a nucleotide
5 reaction mixture consisting of X, Y, and Z, wherein X and Y are different purine non-terminator nucleotides, and Z is a pyrimidine non-terminator nucleotide, or X and Y are different pyrimidine non-terminator nucleotides, and Z is a purine non-terminator nucleotide. A portion of the extension products are hybridized to a second primer that is immobilized on a solid support, and the second primer is extended with at least one nucleotide having a detectable marker. The
10 signal from the detectable marker can be correlated to the amount of target polynucleotide. Kits for performing this method are also provided.